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CELL-MATRIX MECHANICAL INTERACTIONS: MODELLING AN ACTIVE, DEFORMABLE CELL ON A HOMOGENEOUS SUBSTRATE IN 3D

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SUMMARY

Based on a particle-based model used to describe initial, passive cell-spreading, a model for endothelial cell active spreading and migration has been developed. To describe the interaction of this cell-model with a homogeneous viscoelastic solid substrate, the latter has been implemented using the non-inertial smoothed-particle hydrodynamics method. The combination of these models is validated using traction force microscopy on individual cells in addition to geometrical measures such as contact area and eccentricity. This mechanistic validated model allows for a detailed computational investigation of cell-matrix interaction in both 2D and 3D environments.

Key words: *active cell spreading, migration, deformable cell model, SPH, traction force microscopy*

1 INTRODUCTION

Angiogenesis, the creation of new blood vessels from existing ones, is an important process in development, wound healing, and during solid tumor development. A crucial process in angiogenesis is the coordinated migration of endothelial cells that form the sprout. To move, cells have to apply (traction) forces on their surrounding extracellular matrix (ECM), which – at least in 3D – also has to be degraded to create space for the growing blood vessel sprout [1]. Sprouting is controlled by a multitude of factors such as VEGF gradients, the behaviour of neighbouring cells [2], as well as the ECM in which the sprout is growing [3].

This work concentrates on the mechanical cell-ECM interactions needed for sprouting, and to that end we investigate the tractions needed for active spreading and migration of human umbilical vein endothelial cells (HUVECs) as a first step towards a validated model. This model, validated at the single-cell scale, is a crucial step towards meaningful modelling of the larger sprouting structure, as a lack of information on the individual cell scale might otherwise lead to many alternative models to explain sprouting behaviour and structures [4].

In *in vitro* experiments, cells are usually seeded on various surrogate "ECM" substrates in order to have control over the material properties such as elasticity, adhesiveness for a given cell type and structure. Most commonly, such materials (e.g. polyacrylamide (PA) or polyethylene glycol gel) have a nano-scale porosity and are homogeneous as well as linear-elastic for typical deformations caused by cells. This has the advantage of not only allowing a high degree of experimental repeatability, but even allows to solve an inverse problem with reasonably high fidelity for the material-displacements to calculate tractions exerted by the cells of the material – a technique called traction force microscopy (TFM) (see also subsection 2.1).

In order to model the relatively large deformations and eventually degradation of this type of matrix, the non-inertial smoothed particle hydrodynamics (NSPH) method is used (see subsection 2.3).

Finally, to mechanistically model cell behaviour during active spreading and migration, the deformable cell model presented in [5] is extended with a model of a lamellipodium that actively applies tractions on the substrate (see subsection 2.2).

2 METHODOLOGY

2.1 Traction force microscopy and HUVEC experiments

In traction force microscopy, the displacement field a cell induces in an *in vitro* surrogate ECM is used to calculate the cell tractions. In the experiments shown in Fig. 1(c), Green Fluorescent Protein (GFP)-transduced HUVECs have been cultured on PA gels coated with fibronectin in EGM2 medium. The single cell's behaviour in the performed experiments is not migratory, but rather the cells undergo continuous shape-changes by extending and retracting protrusions, thereby actively spreading out on the substrate to a large extent.

2.2 Deformable cell model

The deformable cell model used here is based on the passive cell-mechanical model presented in [5]. Briefly, the cell boundary is discretised into triangles, which allows a mechanical description of the (coarse grained) cortex as well as accurate calculation of both repulsive as adhesive and frictional contact forces with the substrate. An overdamped equation of motion can be obtained by collecting all (fluid)-frictional terms on one side and all conservative forces on the other, which allows to (iteratively) solve for the velocities, which are then integrated by a simple forward-Euler scheme, here written for node i in the triangulated mesh:

$$\begin{aligned}&\sum_{\text{triangles } l}\left[\mathbf{F}^{il}_{\text{contact}}+\mathbf{F}^{il}_{A,\text{local}}+\mathbf{F}^{il}_{\text{lamellipod}}\right]+\sum_{\text{conn. } k}\left[\mathbf{F}^{ik}_{\text{cortex}}+\mathbf{F}^{ik}_{\text{bend}}\right]+\mathbf{F}^{i}_{\text{volume}}+\mathbf{F}^{il}_{A,\text{global}}\\ &=\sum_{\text{triangles } l}\Gamma^{il}_{\text{substrate}}\mathbf{v}^i+\sum_{\text{conn. } k}\gamma(\mathbf{v}^i-\mathbf{v}^k)+\Gamma^{i}_{\text{liquid}}\mathbf{v}^i,\end{aligned}\tag{1}$$

where $\mathbf{F}^{il}_{\text{contact}}$ and $\mathbf{F}^{il}_{A,\text{local}}$ are, respectively, the local (membrane-) area-conservation and contact forces calculated for all triangles adjacent to i. Secondly, the forces representing the cortex-mechanics by stretch ($\mathbf{F}^{ik}_{\text{cortex}}$) and local curvature/bending ($\mathbf{F}^{ik}_{\text{bend}}$) are summed over all connections of node i, and finally forces from a global volume- ($\mathbf{F}^{i}_{\text{volume}}$) and surface area ($\mathbf{F}^{il}_{A,\text{global}}$) conservation are apportioned to each node. The friction forces can be equivalently calculated from the frictions at the adjacent triangles $\Gamma^{il}_{\text{substrate}}\mathbf{v}^i$, the relative motion in the cortex $\gamma(\mathbf{v}^i-\mathbf{v}^k)$, and a fluid drag $\Gamma^{i}_{\text{liquid}}\mathbf{v}^i$.

This model is extended by adding an active lamellipodium by defining a region in the cell periphery which actively applies a uniform traction (subsequently calculated to a force $\mathbf{F}^{i}_{\text{lamellipod}}$ on node i) on the substrate. This region is selected by a (random) polarisation vector, a distance from the cell's centre (nucleus), and the fact that it has to be in contact with the substrate. If the cell's polarisation vector changes relatively rapidly and the region where the propulsive tractions are applied is chosen to be narrow, several distinct protrusions can be obtained at the same time due to the frictional interaction with the substrate. This allows to mimic "active cell spreading" as well as – if the re-polarisation is less frequent – continuous locomotion.

2.3 Model of the substrate

Smoothed particle hydrodynamics (SPH) is a mesh-free Lagrangian method to describe fluid flows or also solids with large deformations. Compared with finite-element methods, it allows a more natural representation of large deformations, modelling of a cell migrating through a matrix without the need for time consuming remeshing and phase transitions to capture matrix degradation (at a typically higher computational cost for a given accuracy). The main idea is to use discrete "particles" to represent the (assumed) homogeneous material, whose properties are "smoothed" out with a kernel

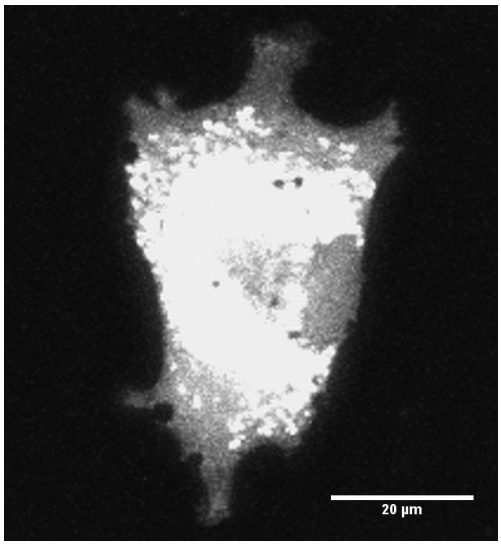
function W with compact support of size h. Then any quantity A at point $\mathbf{r}$ can be calculated as:

$$A(\mathbf{r}) = \sum_j m_j \frac{A_j}{\rho_j} W(|\mathbf{r} - \mathbf{r}_j|, h), \tag{2}$$

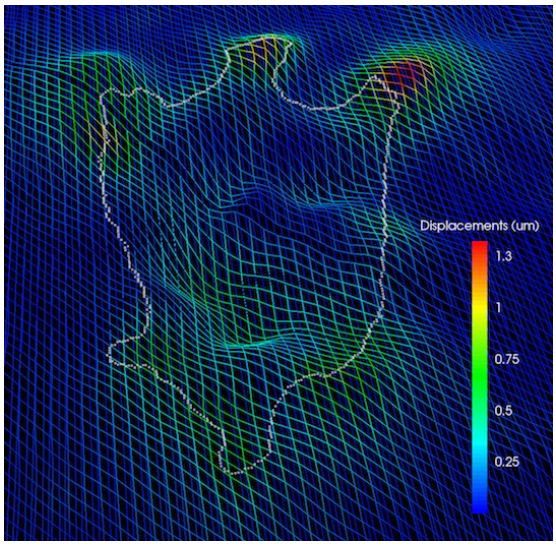
where m_j and ρ_j are the mass and density of particle j. In [6] the method is extended to explicitly neglect inertia in flows (NSPH), yielding a large gain in stability and speed-up for calculations at low Reynolds numbers. In turn, in this work NSPH has been extended with a standard formulation of a viscoelastic solid to compute the response of a typical surrogate extracellular matrix.

3 RESULTS AND CONCLUSIONS

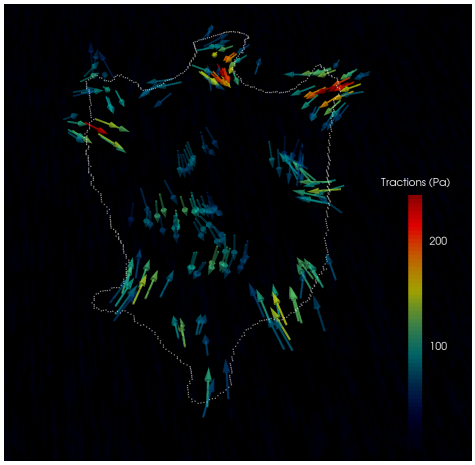
Using the dynamical production rate of protrusions, 3D cell size as well as the properties of the ECM we construct realistic simulations of the actively spreading cell using the methods briefly detailed above. This model can now be statistically compared to e.g. contact area $\mathcal{A}_{\text{contact}}$ as well as traction magnitudes and directions to validate the single-cell mechanical model. The comparison of typical tractions in both model and experiments is shown in Fig. 1.



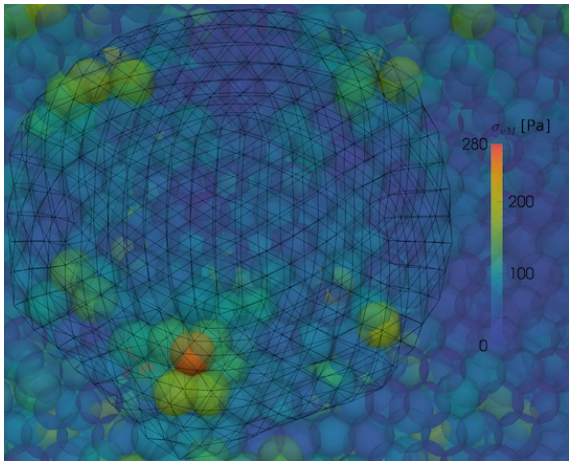
(a) GFP-transduced HUVEC on a PA gel.



(b) Measured displacement field for cell shown in Fig. (a). Cell contour is shown as well.



(c) Measured cellular tractions (by means of TFM) for cell shown in Fig. (a). Cell contour is shown.



(d) Von Mises stress in substrate by simulated cell (shown as wireframe)

Figure 1: Microscopic view of HUVEC on PA substrate (a), as well as the displacement field (b) and recovered tractions on the substrate (c), where the direction is indicated by the arrows and the magnitude by the colour of the arrows. For comparison, a typical simulation example with simulated (von Mises) stresses (d) is shown.

The magnitudes of the experimentally obtained tractions correspond to the calculated von Mises stresses in the substrate. It can be observed that in the simulations residual stresses from several (former) protrusions are visible, which is comparable to the dominant areas of applied tractions in the experimental results (compare Fig. 1, (c) and (d)). To achieve a fully validated model, further statistical comparisons are needed.

The observed displacements and stresses within in the surrogate ECM allow also to on the one hand cross-validate the elastic-NSPH model used. On the other hand, this modelling effort allows for realistic tests of TFM accuracy given a complex set of tractions applied by the simulated cell (and their corresponding displacements) to back-calculate tractions.

The presented methodology allows to validate a dynamic 3D cell-mechanical model and thereby conclusively describe the cell's properties on an elastic adhesive substrate in typical culture conditions. This model can now be used to explore cell behaviour and arrive at specific hypotheses which can be experimentally tested.

4 ACKNOWLEDGEMENTS

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